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$InsP_3$ Triggered Calcium Release Events in Mouse Atrial Myocytes

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In the heart, the dominant mechanism of intracellular Ca^{2+} release is Ca^{2+}-induced Ca^{2+} release (CICR) via sarcoplasmic reticulum (SR) Ca^{2+} release channels (RyR's). Recently, a second mechanism, Ca^{2+} release through channels sensitive to the intracellular second messenger Inositol-1,4,5-trisphosphate ($InsP_3$) has been described. The contribution and significance of $InsP_3$-induced Ca^{2+} release in cardiac excitation-contraction coupling (EC-coupling) is still a matter of debate. However, recent reports have emphasized the importance of $InsP_3$ signaling for EC-coupling in atrial myocytes, for excitation transcription coupling and embryogenesis. Investigating $InsP_3$ signaling is challenging because adequately selective pharmacological tools or fluorescent indicators are not available and the specific activation of highly localized intracellular $InsP_3$ receptors ($InsP_3R$) is hampered due to experimental inaccessibility. We are using UV-flash uncaging approaches of caged $InsP_3$ to study the interplay of $InsP_3R$ Ca^{2+} release and CICR based on the activation of RyR under whole-cell conditions. UV-flash photolysis of caged $InsP_3$ was accompanied by an increase in the number of local Ca^{2+} release events that show larger FDHM and/or smaller amplitude. In the presence of $InsP_3R$ blocker xestospongin C the frequency of $InsP_3$ evoked Ca^{2+} events was reduced and suggest coexistence of spontaneous SR-Ca^{2+} release events (Ca^{2+} sparks) and $InsP_3$ evoked SR-Ca^{2+} release events (Ca^{2+} puffs). In addition, photorelease of $InsP_3$ in PM loaded cells induced global Ca^{2+} release events suggesting that $InsP_3$ facilitation of Ca^{2+} release may be linked to RyR mediated Ca^{2+} release in atrial cells. Two-photon excitation photolysis (TPP) of caged $InsP_3$ was used to study $InsP_3$ signaling in a highly targeted way. TPP triggered $InsP_3$-induced Ca^{2+} release exhibiting spatio-temporal characteristics corresponding to elementary Ca^{2+} signals, such as Ca^{2+} waves and Ca^{2+} ''puffs'' and seems to be promising tools for studying $InsP_3$ signaling on the sub-cellular scale. Supported by SNF.

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Effects of Mitochondrial Membrane Depolarization on Cellular Function in Cardiac Myocytes

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Mitochondria are the primary sites of ATP generation in heart cells. The voltage gradient across the mitochondrial inner membrane ($\Delta\Psi_{mito}$) is a critical feature and this in turn is controlled in part by the mitochondria permeability transition pore (mPTP), whose molecular identity remains elusive. Here we used used illumination-dependent subcellular mitochondrial depolarization to investigate cardiac mitochondrial function in cells exposed to low (nM) concentrations of the fluorescent mitochondrial reporter tetramethyl rhodamine methyl ester (TMRM). The relationship between mPTP gating (as measured by $\Delta\Psi_{mito}$ depolarization) and subcellular myocyte function was examined in single cardiac myocytes. ROS production was measured using dichlorofluorescin (DCF). $[Ca^{2+}]_i$ was measured with intracellular fluo-4. There was a time-dependent increase in the depolarization of those mitochondria exposed to visible light but nearby mitochondria in the same cell but kept in the dark remained normally polarized. Only rarely did the illuminated and depolarized mitochondria repolarize following the cessation of illumination. We have also investigated the hypothesis that the illuminated mitochondrial depolarization is due to a ROS-dependent mechanism. How Ca^{2+} signaling, ROS, $\Delta\Psi_{mito}$ are inter-related will be discussed. Additionally the mitochondrial depolarization dependent actions on other myocyte functions (contraction, $[Ca^{2+}]_i$ transients, Ca^{2+} instability, membrane currents) will be discussed.

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A Comparative Assessment of Fluo Ca^{2+} Indicators in Rat Ventricular Myocytes

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Improvements in the fluo series of fluorescent Ca^{2+} indictors routinely used to measure cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) have increased signal-to-noise ratio, enabling more quantitative $[Ca^{2+}]$ measurements. The improved fluo derivatives show increased cellular loading efficiency, reduced pH sensitivity, and excitation maxima that better match the wavelengths of common lasers. Nevertheless, the extent to which these indicators interfere with native intracellular Ca^{2+} homeostasis has not been systematically characterized. Here, we have examined three different fluo derivatives (fluo-2, fluo-3 and fluo-4) in freshly isolated rat ventricular myocytes. Cells were loaded with a fluo indicator either by incubation with the acetoxymethyl (AM) ester or by introducing the K^+ salt of the indicator through a whole-cell pipette (injection-loaded).

Fluorescence changes in cardiomyocytes were measured using confocal microscopy during field simulation or current injection. Three significant differences were identified among the three indicators and two loading methods. 1. Ca^{2+} kinetics in AM-loaded cells were slower than in injection-loaded cells; thus the decay of the cardiac $[Ca^{2+}]_i$ transient appeared to be slower. In AM-loaded cells, fluo-3 reported the fastest response while fluo-2 and fluo-4 gave comparable, slower responses. When injection-loaded as the K^+ salt, all three indicators reported comparable responses that were faster than any of the AM-loaded indicators. 2. When AM-loaded, all three indicators showed apparent spatial inhomogeneities in cellular fluorescence, in contrast to injection-loaded cells. Thus diverse cellular structures appear brighter in AM-loaded cells than in injection-loaded cells. 3. For each indicator, the calibrated signals (F/F_o) in the AM-loaded cells were higher than in injection-loaded cells.

We conclude that injection-loaded cells produce more accurate $[Ca^{2+}]_i$ measurements and spatially resolved signals. If AM loading is needed, the use of fluo-3 would appear to provide more accurate calibrated signals.

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Impaired Local Calcium Signaling in Primary Cultured Adult Rat Ventricular Myocytes

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Although cultured adult cardiac myocytes have been adopted in studying protein functions in combination with cell-level genetic modifications, cellular alterations by culturing itself need to be clarified to understand real function of the protein genetically altered. We systematically compared contractile properties, calcium ion (Ca^{2+}) signaling, transverse (t)-tubules, ryanodine receptor distributions between freshly isolated and two-days cultured adult rat ventricular myocytes. Density of t-tubules was remarkably decreased by culture. In cultured myocytes, cell shortenings were attenuated by ~60% and relaxation was slowed. Consistently, magnitudes of action potential-induced Ca^{2+} transients were decreased to ~50% and decays of the Ca^{2+} transients were retarded by culture. In cultured cells, density of L-type Ca^{2+} current was reduced to ~40% and its inactivation was retarded. The latter is consistent with smaller Ca^{2+} transients in cultured group. However, sarcoplasmic reticulum Ca^{2+} contents were not different between two groups. To know the mechanism for smaller Ca^{2+} transient in cultured cells we examined Ca^{2+} sparks in these two groups of cells. The frequency of spontaneous Ca^{2+} sparks was significantly decreased by culturing. The amplitude, duration, and time-to-peak of individual Ca^{2+} sparks were not different between the two groups. Mean spark width was two-fold larger in cultured cells compared with freshly isolated cells. Quantitative analysis of immunofluorescence revealed shortening of longitudinal spacing between RyR2 clusters, and less dense and disorganized distributions of RyR2 clusters in cultured cells, which may be related to lower frequency of sparks in these cells. These results provide evidence on significant difference in Ca^{2+} sparks as well as excitation-contraction coupling in primary cultured adult ventricular myocytes. (This work was supported by National Research Foundation of Korea grants funded by the Ministry of Education, Science and Technolgy (2010-0000070).)

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A Caveolin Targeted L-type Calcium Channel Antagonist Inhibits Hypertrophic Signaling without Reducing Contractility of Cardiac Myocytes

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The source of Ca^{2+} to activate pathological hypertrophy is not thought to involve the $[Ca^{2+}]$ that activates contraction. We hypothesize that Ca^{2+} influx through a subpopulation of L-type Ca^{2+} channels ($Ca_V1.2$; $I_{Ca,L}$) localized in caveolin (Cav) containing membrane signaling microdomains locally activates calcium/calmodulin and calcineurin-mediated NFAT nuclear translocation to induce hypertrophy. Rem-GTPase is known to inhibit $Ca_V1.2$ which requires a membrane association c-terminal. We truncated the c-terminal of Rem (Rem^{1-265}) which eliminated $Ca_V1.2$ inhibition and fused Rem^{1-265} to a canonical caveolin binding domain to create Rem^{1-265}-Cav. **Results**: Adenoviral-mediated expression of normal Rem in adult feline myocytes almost fully eliminated $I_{Ca,L}$ while non-membrane targeted Rem^{1-265} had no significant effect on $I_{Ca,L}$. Rem^{1-265}-Cav exhibited a small inhibition (less than 15%) of the normal $I_{Ca,L}$. Myocytes expressing Rem^{1-265}-Cav responded normally to isoproterenol while those infected with normal Rem failed to respond. Myocytes infected with normal Rem had markedly reduced fractional shortening (2.4+/-0.7% resting cell length) while those infected with the truncated Rem^{1-265} (7.8+/-1.8) and Rem^{1-265}-Cav (5.9+/-1.4) had contractions not significantly smaller than controls (7.4+/-2.1). Sucrose density gradient experiments revealed that Rem^{1-265}-Cav cosedimented with caveolin-3 enriched low-density fractions. These experiments suggest Rem^{1-265}-Cav inhibits caveolin-associated $Ca_V1.2$. To investigate the effects of our Rem constructs on NFAT nuclear translocation (hypertrophic signaling) myocytes were also infected with NFAT-GFP. Bath $[Ca^{2+}]$ was elevated to induce NFAT nuclear translocation measured by nuclear to cytoplasmic NFAT-GFP ratio. Normal